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Cytokinesis: Keeping Ring and Membrane Together

During cytokinesis, the actomyosin contractile ring drives ingression of the overlying plasma membrane. A recent study has provided mechanistic insight into how the contractile ring might contribute to membrane ingression.

Manuel Mendoza and Yves Barral

Cytokinesis, the division of one cell into two, initiates towards the end of mitosis, when the plasma membrane invaginates between the segregating chromosomes [1–3]. The region of the membrane undergoing this deformation, known as the cleavage furrow, is tightly associated with the contractile ring, a sub-cortical meshwork of actin and myosin filaments. In most animal cells, furrow ingression depends on actomyosin ring contraction, and extensive analysis of ring components has shed light on the mechanism of ring assembly and contraction (for reviews, see [1–4]). Surprisingly, however, we know very little about how ring dynamics are coupled to changes in membrane shape during cytokinesis. For example, how does the ring associate with the membrane? What is the relationship between ring-generated forces and membrane deformation? New insights into these questions have been provided by the recent identification of a budding yeast protein that couples membrane ingression to ring contraction [5].

Since the discovery 12 years ago that budding yeast, like animal cells, assemble an actomyosin contractile ring during cytokinesis [6,7], it is now accepted that yeast and animal cell-division machineries have many similarities. In the recent work, Sanchez-Diaz and co-workers [5] identified Inn1 (*in*gressio*n 1*), a novel component of the yeast contractile ring required for cytokinesis. Analysis of Inn1-depleted cells revealed a striking phenotype: unlike any other cytokinesis mutant known so far, inactivation of Inn1 causes the actomyosin ring to detach from the plasma membrane upon contraction. Rings lacking Inn1 undergo normal contraction, but the membrane fails to invaginate. Inn1 therefore plays a crucial role in the coupling of membrane ingression and actomyosin contractility.

So, how does Inn1 couple ingression of the plasma membrane to actomyosin ring contraction? The amino-terminal region of Inn1 is predicted to form a C2 domain, a protein fold known to bind biological membranes [8]. The remainder of the protein is rich in PXXP motifs, which are often sites of protein–protein interactions. The study by Sanchez-Diaz *et al.* [5] suggests that Inn1 physically links the membrane and the contractile ring, with its C2 domain binding to the plasma membrane and the rest of the protein anchoring Inn1 to the ring (Figure 1), a model supported by various findings. Localization of Inn1to the site of cell division depends on ring assembly, and the protein physically interacts with the ring components Hof1 and Iqg1. Furthermore, deletion of the C2 domain, or point mutations that disrupt C2-domain-dependent interactions, do not abolish the localization of Inn1 mutant proteins to the cleavage site, but impair cytokinesis. It is therefore likely that Inn1 localizes to the division site through direct association with the ring and that the C2 domain is required for association with the membrane. To directly assess whether Inn1 couples membrane deformation to actin-ring contraction through its C2 domain, the authors targeted the C2 domain to the furrow by fusing it to the ring component Hof1. This C2–Hof1 fusion protein completely rescued cytokinesis in *inn1*Δ cells. Similar results were obtained by fusing the C2 domain of Inn1 to Myo1, the yeast myosin II motor. Inn1 therefore couples ring contraction and membrane ingression, apparently by directly linking the ring with the plasma membrane.

Does Inn1 act as ‘molecular velcro’ attaching the plasma membrane to the contractile ring? The reality seems to be more complicated. Correct positioning, assembly and contractility of the ring do not require Inn1, which is incorporated in the ring shortly before contraction. Thus, the initial association between ring and membrane must depend on factors other than Inn1. Indeed, multiple lipid-binding proteins associate with the division site [9] and could contribute to this initial membrane attachment. So why is Inn1 essential

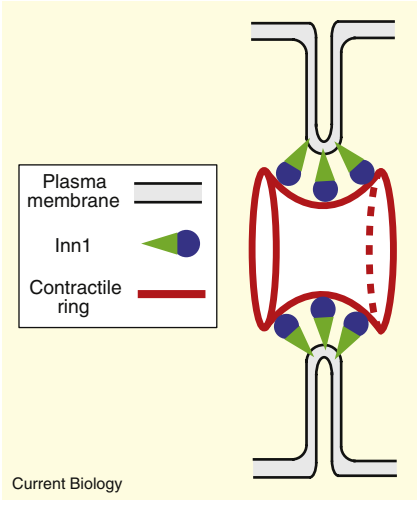

Figure 1. Schematic representation of how Inn1 couples actomyosin ring contraction to membrane ingression during cytokinesis.
Inn1 associates with the plasma membrane through its amino-terminal C2 domain (represented in green) whereas its carboxy-terminal portion (in blue) binds the contractile ring.

to maintain ring association with the membrane specifically during contraction? Since considerable stress is exerted on the membrane during furrowing [10], Inn1 might alter plasma membrane properties, like viscosity or curvature, to promote membrane deformation and to stabilize membrane–ring interactions specifically during furrow ingression. Insertion of the Inn1 C2 domain into the lipid bilayer could have such an effect on the cleavage furrow. In a related case, insertion of the C2 domain of the human vesicle fusion protein synaptotagmin [11] leads to increased membrane curvature. Is regulation of membrane curvature important for furrow ingression? Further studies aimed at characterizing the interaction of Inn1 with membranes should help to answer this question.

If Inn1 were indeed actively involved in membrane remodeling, it would be interesting to address whether it also acts in abscission, when the plasma membrane undergoes the dramatic transition from continuous lipid bilayer into two distinct membranes. This is a poorly understood process in which ring disassembly and vesicle fusion events may play an important role [2,12]. In this context, the observation by Sanchez-Diaz *et al.* [5] that actomyosin ring disassembly is delayed in Inn1-depleted cells is intriguing and could indicate that ring disassembly and abscission are coordinated by a membrane-bound factor, perhaps Inn1 itself.

This recent work [5] identifies Inn1 as the first factor known to link the actomyosin ring to plasma membrane ingression. Although animal cells have no obvious Inn1 homologues, C2 domains are ubiquitous in eukaryotes. It is likely that C2-containing proteins will turn out to be important players in cytokinesis in animal cells. At the last count, more than 270 human proteins were annotated as possessing C2 domains, many of them of unknown function. The hunt is on.

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Behavioural Genetics: Worms Seek That Old Beetle Smell

Some nematodes eavesdrop on pheromonal signals to sniff out their elderly beetle hosts. This turns out to be yet another behaviour regulated by cGMP/PKG signalling.

Marla B. Sokolowski[1] and Mark J. Fitzpatrick[2]

Like vultures of the undergrowth, some nematodes lurk in the soil searching for over-the-hill hosts. They climb aboard an unsuspecting beetle and enter a hibernation-like state until the host dies. Because the nematodes do not appear to harm the living host, they are not parasites; rather, upon the death of the host, the nematodes reawaken to feed on the microorganisms found in the carcass. Fascinating new investigations of the chemosensory aspects of this necromenic lifestyle are described in two recent papers by Hong *et al.* [1,2].

Necromeny in *Pristionchus* nematodes is a very recent discovery [3,4]. These worms exhibit chemoattraction to insect sex pheromones as well as to extracts from plant compounds [1]. They have evolved to intercept the chemical communication system of their insect hosts. In one scenario, the scarab beetle host lives about three years as a larva and pupa, but only three weeks as a feeding adult. The shorter-lived nematodes have evolved to specifically recognize the older feeding beetles, their preferred targets, and Hong *et al.* [1] show that beetle chemosensory cues provide the necessary information. The species *P. maupasi* is most attracted to a cocktail from late-stage feeding beetles, which move repeatedly between soil and foliage, and are generally only weeks from death. In this case, the pheromone and plant volatiles associated with feeding beetles act synergistically to increase chemoattraction of this nematode species. This has two interesting consequences: first, the use of sex pheromones as an attractant ensures that the worms infect the proper species, and only the adults so that they avoid getting 'trapped' for years on a larva or pupa; and second, the synergistic attractant effects of the plant volatiles may help the worms identify hosts who have made more feeding forays, and are therefore older and closer to death.